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General information about the entry

Entry name	BXB_CLOBO
Primary accession number	P10844
Secondary accession number	P10843
Entered in SWISS-PROT in	Release 11, July 1989
Sequence was last modified in	Release 26, July 1993
Annotations were last modified in	Release 41, June 2002

Name and origin of the protein

Protein name	Botulinum neurotoxin type B [Precursor]
Synonyms	EC <u>3.4.24.69</u> BoNT/B Bontoxilysin B
Gene name	BOTB
From	<u>Clostridium botulinum</u> [TaxID: <u>1491</u>]
Taxonomy	<u>Bacteria</u> ; <u>Firmicutes</u> ; <u>Clostridia</u> ; <u>Clostridiales</u> ; <u>Clostridiaceae</u> ; <u>Clostridium</u> .

References

- [1] SEQUENCE FROM NUCLEIC ACID.
MEDLINE=92384550; PubMed=1514783; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]
[Whelan S.M.](#), [Elmore M.J.](#), [Bodsworth N.J.](#), [Brehm J.K.](#), [Atkinson T.](#), [Minton N.P.](#);
"Molecular cloning of the Clostridium botulinum structural gene encoding the type B neurotoxin and determination of its entire nucleotide sequence.";
[Appl. Environ. Microbiol.](#) 58:2345-2354(1992).
- [2] SEQUENCE OF 35-245 FROM NUCLEIC ACID.
STRAIN=NCTC 7273;
[Szabo E.A.](#), [Pemberton J.M.](#), [Desmarchelier P.M.](#);
Submitted (APR-1992) to the EMBL/GenBank/DDBJ databases.
- [3] SEQUENCE OF 633-993 FROM NUCLEIC ACID.
STRAIN=NCTC 7273;
MEDLINE=94013372; PubMed=8408542; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]
[Campbell K.](#), [East A.K.](#), [Collins M.D.](#);
"Gene probes for identification of the botulinal neurotoxin gene and specific identification of neurotoxin types B, E, and F.";
[J. Clin. Microbiol.](#) 31:2255-2262(1993).
- [4] SEQUENCE OF 1-44 AND 441-466.
STRAIN=657;
MEDLINE=89000987; PubMed=3139097; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]
[Dasgupta B.R.](#), [Datta A.](#);
"Botulinum neurotoxin type B (strain 657): partial sequence and similarity with tetanus toxin.";
[Biochimie](#) 70:811-817(1988).
- [5] SEQUENCE OF 1-16 AND 441-458.
STRAIN=OKRA;
MEDLINE=85197963; PubMed=3888113; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]

"	<p><u>Schmidt J.J., Sathyamoorthy V., Dasgupta B.R.:</u> "Partial amino acid sequences of botulinum neurotoxins types B and E." Arch. Biochem. Biophys. 238:544-548(1985).</p>
[6]	<p>IDENTIFICATION AS ZINC-PROTEASE. MEDLINE=93054694; PubMed=1429690; [NCBI, ExpASY, EBI, Israel, Japan] <u>Schiavo G., Rossetto O., Santucci A., Dasgupta B.R., Montecucco C.:</u> "Botulinum neurotoxins are zinc proteins." J. Biol. Chem. 267:23479-23483(1992).</p>
[7]	<p>IDENTIFICATION OF SUBSTRATE. MEDLINE=93063293; PubMed=1331807; [NCBI, ExpASY, EBI, Israel, Japan] <u>Schiavo G., Benfenati F., Poulain B., Rossetto O., de Laureto P.P., Dasgupta B.R., Montecucco C.:</u> "Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin." Nature 359:832-835(1992).</p>

Comments

FUNCTION: BOTULINUS TOXIN ACTS BY INHIBITING NEUROTRANSMITTER RELEASE. IT BINDS TO PERIPHERAL NEURONAL SYNAPSES, IS INTERNALIZED AND MOVES BY RETROGRADE TRANSPORT UP THE AXON INTO THE SPINAL CORD WHERE IT CAN MOVE BETWEEN POSTSYNAPTIC AND PRESYNAPTIC NEURONS. IT INHIBITS NEUROTRANSMITTER RELEASE BY ACTING AS A ZINC ENDOPEPTIDASE THAT CLEAVES THE 76-GLN-|-PHE-77 BOND OF SYNAPTOBREVIN-2.

CATALYTIC ACTIVITY: Limited hydrolysis of proteins of the neuroexocytosis apparatus, synaptobrevins, SNAP25 or syntaxin. No detected action on small molecule substrates.

COFACTOR: Binds 1 zinc ion per subunit (*By similarity*).

SUBUNIT: DISULFIDE-LINKED HETERODIMER OF A LIGHT CHAIN (L) AND A HEAVY CHAIN (H). THE LIGHT CHAIN HAS THE PHARMACOLOGICAL ACTIVITY, WHILE THE N-AND C-TERMINAL OF THE HEAVY CHAIN MEDiate CHANNEL FORMATION AND TOXIN BINDING, RESPECTIVELY.

SUBCELLULAR LOCATION: Secreted.

MISCELLANEOUS: THERE ARE SEVEN ANTIGENICALLY DISTINCT FORMS OF BOTULINUM NEUROTOXIN: TYPES A, B, C1, D, E, F, AND G.

SIMILARITY: BELONGS TO PEPTIDASE FAMILY M27.

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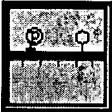
EMBL	M81186; AAA23211.1; [EMBL / GenBank / DDBJ] -. [CoDingSequence] Z11934; CAA77991.1; [EMBL / GenBank / DDBJ] -. [CoDingSequence] X70817; CAA50148.1; [EMBL / GenBank / DDBJ] -. [CoDingSequence]
PIR	S07128; S07128. S07155; S07155. S08562; S08562. S08573; S08573. S08574; S08574. A48940; A48940.
HSSP	P10845; 3BTA. [HSSP ENTRY / PDB]
MEROPS	M27.002; -.
InterPro	IPR000395; Bontoxilysin. IPR000130; Zn_MTpeptdse. Graphical view of domain structure.
Pfam	PF01742; Peptidase_M27; 1.
PRINTS	PR00760; BONTOTOXILYSIN.
ProDom	PD001963; Bontoxilysin; 1. [Domain structure / List of seq. sharing at least 1 domain] .
PROSITE	PS00142; ZINC_PROTEASE; 1.
BLOCKS	P10844

BLOCKS	P10844.
ProtoNet	P10844.
ProtoMap	P10844.
PRESAGE	P10844.
DIP	P10844.
ModBase	P10844.
SWISS-2DPAGE	GET REGION ON 2D PAGE.

Keywords

Neurotoxin; Transmembrane; Hydrolase; Metalloprotease; Zinc.

Features

Key	From	To	Length	Description	
INIT_MET	0	0			
CHAIN	1	440	440	BOTULINUM NEUROTOXIN B, LIGHT-CHAIN.	
CHAIN	441	1290	850	BOTULINUM NEUROTOXIN B, HEAVY-CHAIN.	
METAL	229	229		ZINC (CATALYTIC) (BY SIMILARITY).	 Feature aligner
ACT_SITE	230	230		BY SIMILARITY.	
METAL	233	233		ZINC (CATALYTIC) (BY SIMILARITY).	 Feature table viewer
DISULFID	436	445		INTERCHAIN (PROBABLE).	
CONFLICT	29	29		T -> M (IN REF. 4).	
CONFLICT	217	217		R -> G (IN REF. 2).	
CONFLICT	224	224		A -> S (IN REF. 2).	
CONFLICT	463	463		S -> R (IN REF. 4).	

Sequence information

Length: 1290 AA [This is the length of the unprocessed precursor]	Molecular weight: 150670 Da [This is the MW of the unprocessed precursor]	CRC64: D21746E2C024DF43 [This is a checksum on the sequence]
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130	140	150	160	170	180

RRVPLEEFNT	NIASVTVNKL	ISNPGEVERK	KGIFANLIIF	GPGPVLNENE	TIDIGIQNHF
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IKVDDLPIVP	NEKKFFMQST	DAIQAEELYT	FGGQDPSIIT	PSTDKSIYDK	VLQNFRGIVD
310	320	330	340	350	360
RLNKVLVCIS	DPNININIK	NKFKDKYKFV	EDSEGKYSID	VESFDKLYKS	LMFGFTETNI
370	380	390	400	410	420
AENYKIKTRA	SYFSDSLPPV	KIKNLLDNEI	YTIEEGFNIS	DKDMEKEYRG	QNKAINKQAY
430	440	450	460	470	480
EEISKEHLAV	YKIQMCKSVK	APGICIDVDN	EDLFFIADKN	SFSDDLKNE	RIEYNTQSNY
490	500	510	520	530	540
IENDFPINEL	ILDIDLISKI	ELPSENTESL	TDFNVDPVPY	EKQPAIKKIF	TDENTIFQYL
550	560	570	580	590	600
YSQTFPLDIR	DISLTSSFDD	ALLFSNKVYS	FFSMDYIKTA	NKVVEAGLFA	GWVKQIVNDF
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VIEANKSNTM	DKIADISLIV	PYIGLALNVG	NETAKGNFEN	AFEIAGASIL	LEFIPELLIP
670	680	690	700	710	720
VVGAFLLSEY	IDNKNKIKT	IDNALTKRNE	KWSDMYGLIV	AQWLSTVNTQ	FYTIKEGMYK
730	740	750	760	770	780
ALNYQAQALE	EIIKYRYNIY	SEKEKSNINI	DFNDINSKLN	EGINQAIDNI	NNFINGCSVS
790	800	810	820	830	840
YLMKKMIPLA	VEKLLDFDNT	LKKNLLNYID	ENKLYLIGSA	EYEKSKVNKY	LKTIMPFDLIS
850	860	870	880	890	900
IYTNDTILIE	MFNKYNSEIL	NNIILNLRYK	DNNLIDLSGY	GAKVEVYDGV	ELNDKNQFKL
910	920	930	940	950	960
TSSANSKIRV	TQNQNIIFNS	VFLDFSVSFW	IRIPKYKNDG	IQNYIHNEYT	IINCMKNNSG
970	980	990	1000	1010	1020
WKISIRGNRI	IWTLIDINGK	TKSVFFEYNI	REDISEYINR	WFFVTITNNL	NNAKIYINGK
1030	1040	1050	1060	1070	1080
LESNTDIKDI	REVIANGEII	FKLDGDIDRT	QFIWMKYFSI	FNTELSQSNI	EERYKIQSYS
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GEKFIIRRKs NSQSINDDIV RKEDYIYLDf FNLNQEWRVY TYKYFKKEEE KLFLAPISDS
      1210      1220      1230      1240      1250      1260
DEFYNTIQIK EYDEQPTYSC QLLFKKDEES TDEIGLIGIH RFYESGIVFE EYKDYFCISK
      1270      1280      1290
WYLKEVKRKP YNLKLGCNWQ FIPKDEGWTE
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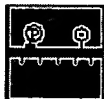
Direct BLAST submission at
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ScanProsite, MotifScan



Sequence analysis tools:
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PeptideCutter, Dotlet (Java)



Feature table viewer (Java)



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[\[Keywords\]](#) [\[Features\]](#) [\[Sequence\]](#) [\[Tools\]](#)

General information about the entry

Entry name	Q9R540
Primary accession number	Q9R540
Secondary accession numbers	None
Entered in TrEMBL in	Release 13, May 2000
Sequence was last modified in	Release 13, May 2000
Annotations were last modified in	Release 15, October 2000

Name and origin of the protein

Protein name	Neurotoxin heavy chain 18 kDa fragment [Fragment]
Synonyms	None
Gene name	None
From	<u>Clostridium botulinum</u> [TaxID: 1491]
Taxonomy	<u>Bacteria</u> ; <u>Firmicutes</u> ; <u>Clostridia</u> ; <u>Clostridiales</u> ; <u>Clostridiaceae</u> ; <u>Clostridium</u> .

References

[1] SEQUENCE.

MEDLINE=94000342; PubMed=8397793; [[NCBI](#), [ExPASy](#), [EBI](#), [Israel](#), [Japan](#)]

[Gimenez J.A.](#), [DasGupta B.R.](#);

"Botulinum type A neurotoxin digested with pepsin yields 132, 97, 72, 45, 42, and 18 kD fragments.";

J. Protein Chem. 12:351-363(1993).

Comments

None

Cross-references

HSSP	P10845 ; 3BTA. [HSSP ENTRY / PDB]
ProDom	[Domain structure / List of seq. sharing at least 1 domain].
ProtoMap	Q9R540 .
PRESAGE	Q9R540 .
ModBase	Q9R540 .
SWISS-2DPAGE	GET REGION ON 2D PAGE .

Keywords

None

Features

None

Sequence information

Length: 72 AA	Molecular weight: 8165 Da	CRC64: B7A959576A615E18 [This is a checksum on the sequence]
<div> <div> 10 IYLNSSLYRG </div> <div> 20 TKFIIKKYAS </div> <div> 30 GNKDNIVRNN </div> <div> 40 DRVYINVVVK </div> <div> 50 NKEYRLATNA </div> <div> 60 SQAGVEKILS </div> </div> <div> 70 ALEIPDVGNL </div> <div> YQ </div>		
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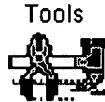
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To: STIC-ILL
Subject: 09/910,186 neurotoxin

Curr Microbiol 1994 Feb;28(2):101-10

Nucleotide sequence of the gene coding for non-proteolytic *Clostridium botulinum* type B neurotoxin:
comparison with other
clostridial neurotoxins.

Hutson RA, Collins MD, East AK, Thompson DE.

Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, UK.

Ginny Portner
CM1, Art Unit 1645
Room 7e13
Mail box 7e12
(703) 308-7543

STIC-ILL

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From: Portner, Ginny
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Appl Environ Microbiol 1993 Sep;59(9):3011-20

Detection of the genes encoding botulinum neurotoxin types A to E by the polymerase chain reaction.

Szabo EA, Pemberton JM, Desmarchelier PM.

Department of Microbiology, University of Queensland, Australia.

Ginny Portner
CM1, Art Unit 1645
Room 7e13
Mail box 7e12
(703) 308-7543

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Imp rtance: High



J Clin Microbiol 1993 Sep;31(9):2255-62

Gene probes for identification of the botulinal neurotoxin gene and specific identification of neurotoxin types B, E, and F.

Campbell KD, Collins MD, East AK.

Reading Laboratory, Institute of Food Research, Agriculture and Food Research Council, United Kingdom.

Ginny Portner
CM1, Art Unit 1645
Room 7e13
Mail box 7e12
(703) 308-7543

Appl. Environ. Microbiol., Aug 1992, 2345-2354, Vol 58, No. 8

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Molecular cloning of the *Clostridium botulinum* structural gene encoding the type B neurotoxin and determination of its entire nucleotide sequence

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SM Whelan, MJ Elmore, NJ Bodsworth, JK Brehm, T Atkinson and NP Minton

Division of Biotechnology, PHLS Centre for Applied Microbiology and Research, Salisbury, Wiltshire, United Kingdom.

DNA fragments derived from the *Clostridium botulinum* type A neurotoxin (BoNT/A) gene (botA) were used in DNA-DNA hybridization reactions to derive a restriction map of the region of the *C. botulinum* type B strain Danish chromosome encoding botB. As the one probe encoded part of the BoNT/A heavy (H) chain and the other encoded part of the light (L) chain, the position and orientation of botB relative to this map were established. The temperature at which hybridization occurred indicated that a higher degree of DNA homology occurred between the two genes in the H-chain-encoding region. By using the derived restriction map data, a 2.1-kb BglII-XbaI fragment encoding the entire BoNT/B L chain and 108 amino acids of the H

Chain was cloned and characterized by nucleotide sequencing. A contiguous 1.8-kb XbaI fragment encoding a further 623 amino acids of the H chain was also cloned. The 3' end of the gene was obtained by cloning a 1.6-kb fragment amplified from genomic DNA by inverse polymerase chain reaction. Translation of the nucleotide sequence derived from all three clones demonstrated that BoNT/B was composed of 1,291 amino acids. Comparative alignment of its sequence with all currently characterized BoNTs (A, C, D, and E) and tetanus toxin (TeTx) showed that a wide variation in percent homology occurred dependent on which component of the dichain was compared. Thus, the L chain of BoNT/B exhibits the greatest degree of homology (50% identity) with the TeTx L chain, whereas its H chain is most homologous (48% identity) with the BoNT/A H chain. Overall, the six neurotoxins were shown to be composed of highly conserved amino acid domains interceded with amino acid tracts exhibiting little overall similarity. In total, 68 amino acids of an average of 442 are absolutely conserved between L chains and 110 of 845 amino acids are conserved between H chains. Conservation of Trp residues (one in the L chain and nine in the H chain) was particularly striking. The most divergent region corresponds to the extreme carboxy terminus of each toxin, which may reflect differences in specificity of binding to neurone acceptor sites.

This article has been cited by other articles:

- Lindstrom, M., Keto, R., Markkula, A., Nevas, M., Hielm, S., Korkeala, H. (2001). Multiplex PCR Assay for Detection and Identification of *Clostridium botulinum* Types A, B, E, and F in Food and Fecal Material. *Appl. Environ. Microbiol.* 67: 5694-5699 [\[Abstract\]](#) [\[Full Text\]](#)
- Kimura, B., Kawasaki, S., Nakano, H., Fujii, T. (2001). Rapid, Quantitative PCR Monitoring of Growth of *Clostridium botulinum* Type E in Modified-Atmosphere-Packaged Fish. *Appl. Environ. Microbiol.* 67: 206-216 [\[Abstract\]](#) [\[Full Text\]](#)
- Hutson, R. A., Zhou, Y., Collins, M. D., Johnson, E. A., Hatheway, C. L., Sugiyama, H. (1996). Genetic Characterization of *Clostridium botulinum*

Type A Containing Silent Type B Neurotoxin Gene Sequences. *J. Biol. Chem.* 271: 10786-10792 [[Abstract](#)] [[Full Text](#)]

Shapiro, R. E., Specht, C. D., Collins, B. E., Woods, A. S., Cotter, R. J., Schnaar, R. L. (1997). Identification of a Ganglioside Recognition Domain of Tetanus Toxin Using a Novel Ganglioside Photoaffinity Ligand. *J. Biol. Chem.* 272: 30380-30386 [[Abstract](#)] [[Full Text](#)]

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☐ 1: J Clin Microbiol 1994
Aug;32(8):1911-7

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Detection of type A, B, and E botulism neurotoxin genes in *Clostridium botulinum* and other *Clostridium* species by PCR: evidence of unexpressed type B toxin genes in type A toxigenic organisms.

PubMed
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Franciosa G, Ferreira JL, Hatheway CL.

Istituto Superiore di Sanita, Laboratorio Alimenti, Roma, Italy.

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We studied the effectiveness of the PCR in detecting type A, B, and E botulism neurotoxin genes in 209 strains of *Clostridium botulinum* and 29 strains of other *Clostridium* spp. All 79 strains that produced type A toxin, 77 strains that produced type B toxin, and 51 organisms that produced type E toxin (46 *C. botulinum* and 5 *C. butyricum*) were positive in reactions with primers targeting sequences specific for their respective toxin genes. The PCR for type A toxin was positive for one type B toxin-producing strain that produced a small amount of type A toxin in addition to a large amount of type B toxin. Surprisingly, the type

toxin gene was detected in addition to the type A toxin gene in 43 type A toxin-producing strains, only 1 of which could be shown by bioassay to produce biologically active type B toxin in culture. The type B gene was also detected in two strains of *C. subterminale*, which were determined to be nontoxigenic by bioassay. While the PCR was sensitive and specific in detecting the neurotoxin genes, the discovery of unexpressed toxin genes indicates that PCR results may not be adequate for establishing type B neurotoxigenicity.

PMID: 7989542 [PubMed - indexed for MEDLINE]

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☐ 1: J Appl Bacteriol 1994
Jun;76(6):539-45

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Polymerase chain reaction for detection of Clostridium botulinum types A, B and E in food, soil and infant faeces.

Szabo EA, Pemberton JM, Gibson AM, Eyles MJ, Desmarchelier PM.

PubMed
Services

Department of Microbiology, University of Queensland
Australia.

Related
Resources

The application of the polymerase chain reaction (PCR) for the detection of Clostridium botulinum types A, B and E in foods, environmental and clinical samples was evaluated and compared to the mouse bioassay. Samples inoculated with 10, 100 and 1000 spores of Cl. botulinum types A and E included pasteurized milk, UHT milk, infant formula, infant faeces, meat juice, canned tuna, mushrooms, blood sausage and soil. Clostridium botulinum type E spores were inoculated into fish eggs, canned tuna, pickled herring, fish and soil at similar levels. Spores were added to 2.5 g of each sample with the exception of soil which was inoculated in 10 g samples. The presence of Cl. botulinum in samples was determined by both PCR and the bioassay.

enrichments was determined by both PCR and the bioassay. An overall correlation of 95.6% was observed between results and the mouse bioassay. Of the total of 114 samples tested there was disparity between the mouse bioassay and the PCR in three samples of soil inoculated with 100 type A or E spores and 10 type B spores per 10 g, respectively, two samples of infant faeces inoculated with 10 type A spores per 2.5 g. All of these samples gave negative assay results and positive PCR results.

PMID: 8027003 [PubMed - indexed for MEDLINE]

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☐ 1: Eur J Biochem 1993 Nov
1;217(3):965-71

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**Proteolytic cleavage of synthetic fragments of
vesicle-associated membrane protein, isoform
by botulinum type B neurotoxin.**

Shone CC, Quinn CP, Wait R, Hallis B, Fooks SG,
Hambleton P.

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Centre for Applied Microbiology and Research, Porton
Down, England.

Related
Resources

Recent data suggest that botulinum type-B neurotoxin
protease which acts on vesicle-associated membrane
protein, isoform 2 (VAMP-2). In this report, botulinum
type-B neurotoxin is shown to cleave a synthetic fragr
(HV62) of VAMP-2, corresponding to the bulk of the
hydrophilic domain (amino acids 33-94). The neurotoxi
at a single site between Gln76 and Phe77. Little or no
proteolytic activity by botulinum type-B neurotoxin wa
observed with peptides containing 7, 10 or 20 amino ac
spanning the site of cleavage. The proteolytic action o
neurotoxin was strongly inhibited by EDTA and
o-phenanthroline whereas captopril and phosphoramid

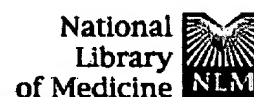
were ineffective. A series of model peptide substrates were synthesised in order to define the smallest VAMP fragment to be cleaved by botulinum type-B neurotoxin. Data obtained from these substrates suggest that the neurotoxin belongs to a novel class of zinc-endopeptidase. More than 12 amino acid residues are required on both NH₂- and COOH-terminal side of the cleavage site for optimal proteolytic activity. The results demonstrate that no other components of cellular vesicles are required for the specific action of the neurotoxin on VAMP-2. The results further show that the highly specific action of the neurotoxin is not dictated solely by the properties of amino acid residues at the cleavage site but is also dependent on amino acid sequences distal to its site of action.

PMID: 8223654 [PubMed - indexed for MEDLINE]

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☐ 1: Appl Environ Microbiol 1993
Sep;59(9):3011-20

Related Art

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PubMed

Detection of the genes encoding botulinum neurotoxin types A to E by the polymerase chain reaction.

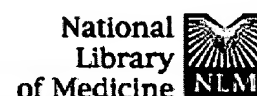
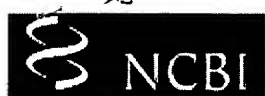
Szabo EA, Pemberton JM, Desmarchelier PM.

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Department of Microbiology, University of Queensland, Australia.

Related
Resources

The polymerase chain reaction (PCR) was used as the basis for the development of highly sensitive and specific diagnostic tests for organisms harboring botulinum neurotoxin type A through E genes. Synthetic DNA primers were selected from nucleic acid sequence data for *Clostridium botulinum* neurotoxins. Individual components of the PCR for each serotype (serotypes A through E) were adjusted for optimal amplification of the target fragment. Each PCR assay was tested with organisms expressing each of the botulinum neurotoxin types (types A through G), *Clostridium tetani*, genetically related nontoxicogenic organisms, and unrelated strains. Each assay was specific for the intended target. The PCR reliably identified multiple strains having the same neurotoxin type. The sensitivity



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1;238(2):544-8Related Art
LEntrez
PubMed**Partial amino acid sequences of botulinum
neurotoxins types B and E.****Schmidt JJ, Sathyamoorthy V, DasGupta BR.**PubMed
Services

Clostridium botulinum type E neurotoxin, a single-chain protein of Mr 147,000, was purified and subjected to acid sequencing. The same was done for single-chain botulinum type B neurotoxin (Mr 152,000), and for the heavy and light chains (Mr 104,000 and 51,000 respectively) derived from type B by limited trypsin digestion. Twelve eighteen residues were identified and the following conclusions were drawn: The light chain of the nicked (dichain) type B is derived from the N-terminal one-third of the single-chain (unnicked) parent neurotoxin; sequence homologies are present between single-chain types B and E and the light chain of the nicked type A [J. J. Schmidt, Sathyamoorthy, and B. R. DasGupta (1984) Biochem. Biophys. Res. Commun. 119, 900-904]; the N-terminal regions of heavy chains of types A and B have some structural similarity; and activation of type B neurotoxin cannot involve removal of amino acids or peptides from the N terminus.

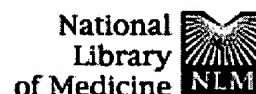
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PMID: 3888113 [PubMed - indexed for MEDLINE]



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Jan;58(1):418-20

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Specific detection of *Clostridium botulinum* type B by using the polymerase chain reaction.

Szabo EA, Pemberton JM, Desmarchelier PM.

Department of Microbiology, University of Queensland, Australia.

PubMed
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The polymerase chain reaction (PCR) and a radiolabeled oligonucleotide probe were used to specifically detect proteolytic and nonproteolytic *Clostridium botulinum* type B. Two synthetic primers deduced from the amino acid sequence data of type B neurotoxin were used to amplify a 1.5-kbp fragment corresponding to the light chain of the toxin. Although, nonspecific priming was observed when the PCR protocol was tested with other clostridial species, the PCR product from *C. botulinum* type B isolates reacted with the radiolabeled internal probe. As little as 100 fg of DNA (approximately 35 clostridial cells) could be detected after only 25 amplification cycles.

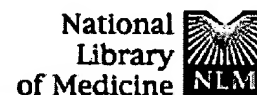
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☐ 1: Adv Exp Med Biol 1996;389:251-60 Related Articles, L

Entrez
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Tetanus and botulism neurotoxins: a novel group of zinc-endopeptidases.

Tonello F, Morante S, Rossetto O, Schiavo G, Montecucco C.

Centro CNR Biomembrane and Dipartimento di Scienze Biomediche, Universita di Padova, Italy.

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Tetanus and botulinum neurotoxins are produced by bacteria of the genus *Clostridium* and cause the paralytic syndrome of tetanus and botulism with a persistent inhibition of neurotransmitter release at central and peripheral synapses respectively. These neurotoxins consist of two disulfide-linked polypeptides: H (100 kDa) is responsible for neurospecific binding and cell penetration and L (50 kDa), a zinc-endopeptidase specific for three protein subunits of the neuroexocytosis apparatus. Tetanus neurotoxin and botulinum neurotoxins serotypes B, D, F, and G cleave at single sites, which differ for each neurotoxin.

VAMP/synaptobrevin, a membrane protein of the synaptic vesicles. Botulinum A and E neurotoxins cleave SNAP-25, a protein of the presynaptic membrane, at two different carboxyl-terminal peptide bonds. Serotype C cleaves

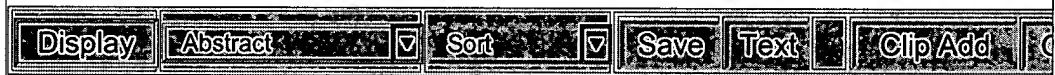
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specifically syntaxin, another protein of the nerve plasmalemma. The target specificity of these metallo-proteinases relies on a double recognition of the substrates based on interactions with the cleavage site with a non contiguous segment that contains a structural motif common to VAMP, SNAP-25 and syntaxin.

Publication Types:

- Review
- Review, Tutorial

PMID: 8861019 [PubMed - indexed for MEDLINE]



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Related Articles, Links

Application of PCR to a clinical and environmental investigation of a case of equine botulism.

Szabo EA, Pemberton JM, Gibson AM, Thomas RJ, Pascoe RR, Desmarchelier PM.

Department of Microbiology, University of Queensland, St. Lucia, Australia.

PCR for the detection of botulinum neurotoxin gene types A to E was used in the investigation of a case of equine botulism. Samples from a foal diagnosed with toxicoinfectious botulism in 1985 were reanalyzed by PCR and the mouse bioassay in conjunction with an environmental survey. Neurotoxin B was detected by mouse bioassay in culture enrichments of serum, spleen, feces, and intestinal contents. PCR results compared well with mouse bioassay results, detecting type B neurotoxin genes in these samples and also in a liver sample. Other neurotoxin types were not detected by either test. *Clostridium botulinum* type B was shown to be prevalent in soils collected from the area in which the foal was raised. Four methods were used to test for the presence of botulinum neurotoxin-producing organisms in 66 soil samples taken within a 5-km radius: PCR and agarose gel electrophoresis (types A to E), PCR and an enzyme-linked assay (type B), hybridization of crude alkaline cell lysates with a type B-specific probe, and the mouse bioassay (all types). Fewer soil samples were positive for *C. botulinum* type B by the mouse bioassay (15%) than by any of the DNA-based detection systems. Hybridization of a type B-specific probe to DNA dot blots (26% of the samples were positive) and PCR-enzyme-linked assay (77% of the samples were positive) were used for the rapid analysis of large numbers of samples, with sensitivity limits of 3×10^6 and 3,000 cells, respectively. Conventional detection of PCR products by gel electrophoresis was the most sensitive method (300-cell limit), and in the present environmental survey, neurotoxin B genes only were detected in 94% of the samples.

PMID: 7989554 [PubMed - indexed for MEDLINE]

Nucleotide sequence of the gene coding for non-proteolytic *Clostridium botulinum* type B neurotoxin: comparison with other clostridial neurotoxins.

Hutson RA, Collins MD, East AK, Thompson DE.

Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, UK.

The neurotoxin gene of non-proteolytic *Clostridium botulinum* type B (strain Eklund 17B) was cloned as a series of overlapping polymerase chain reaction (PCR) fragments generated with primers designed to conserved regions of published botulinal toxin (BoNT) sequences. The 3' end of the gene was obtained by using primers designed to the determined sequence of non-proteolytic BoNT/B and a published downstream region of BoNT/B gene from a proteolytic strain. Translation of the nucleotide sequence derived from cloned PCR fragments demonstrated the toxin gene encodes a protein of 1291 amino acid residues. Comparative alignment of the derived BoNT/B sequence with those of other published botulinal neurotoxins revealed highest sequence relatedness with BoNT/B of proteolytic *C. botulinum*.

The sequence identity between non-proteolytic and proteolytic BoNT/B was 97.7% for the light chain (corresponding to 10 amino acid changes) and 90.2% for the heavy chain (corresponding to 81 amino acid changes), with most differences occurring at the C-terminal end. A genealogical tree constructed from all known botulinal neurotoxin sequences revealed marked topological differences with a phylogenetic tree of *C. botulinum* types based upon small-subunit (16S) ribosomal RNA sequences.

Biochim Biophys Acta 1993 Dec 14;1216(3):487-91

Nucleotide sequence of the gene coding for *Clostridium botulinum* (*Clostridium argentinense*) type G neurotoxin: genealogical comparison with other clostridial neurotoxins.

Campbell K, Collins MD, East AK.

Department of Microbiology, Institute of Food Research, Reading Laboratory, Earley Gate, UK.

The neurotoxin gene from *Clostridium botulinum* type G was cloned as a series of overlapping DNA fragments generated using polymerase chain reaction (PCR) technology and primers designed to conserved regions of published botulinal toxin (BoNT) sequences. The 5'-end of the gene was obtained using a primer based on a conserved region of the nontoxic-nonhaemagglutinin gene lying upstream of the toxin gene. Translation of the nucleotide sequence derived from the cloned PCR fragments demonstrated that the gene encodes a protein of 1297 amino acid residues (rmm 149, 147). Comparative alignment of the determined BoNT/G sequence with those of other clostridial neurotoxins revealed highest sequence relatedness (approx. 58% amino acid identity) with BoNT/B of proteolytic and non-proteolytic *C. botulinum*. Tetanus toxin (TeTx) and other BoNT types revealed lower levels of relatedness with BoNT/G (approximate range 35-42% amino acid identity).

PMID: 8268233 [PubMed - indexed for MEDLINE]

Related Articles, Links

Bacterial extracellular zinc-containing metalloproteases.

Hase CC, Finkelstein RA.

Department of Molecular Microbiology and Immunology, School of Medicine,
University of Missouri, Columbia 65212.

Extracellular zinc-containing metalloproteases are widely distributed in the bacterial world. The most extensively studied are those which are associated with pathogenic bacteria or bacteria which have industrial significance. They are found practically wherever they are sought in both gram-negative and gram-positive microorganisms, be they aerobic or anaerobic. This ubiquity in itself implies that these enzymes serve important functions for the organisms which produce them.

Because of the importance of zinc to enzymatic activity, it is not surprising that there is a pervasive amino acid sequence homology in the primary structure of this family of enzymes regardless of their source. The evidence suggests that both convergent and divergent evolutionary forces are at work. Within the large family of bacterial zinc-containing metalloendopeptidases, smaller family units are observed, such as thermolysin-like, elastase-like, and Serratia protease-like metalloproteases from various bacterial species. While this review was in the process of construction, a new function for zinc-containing metalloproteases was discovered: the neurotoxins of *Clostridium tetani* and *Clostridium botulinum* type B have been shown to be zinc metalloproteases with specificity for synaptobrevin, an integral membrane protein of small synaptic vesicles which is involved in neurotransmission. Additional understanding of the mode of action of proteases which contribute to pathogenicity could lead to the development of inhibitors, such as chelators, surrogate substrates, or antibodies, which could prevent or interrupt the disease process. Further studies of this broad family of metalloproteases will provide important additional insights into the pathogenesis and structure-function relationships of enzymes and will lead to the development of products, including "designer proteins," which might be industrially and/or therapeutically useful.

Publication Types:

Review

Review, Academic

PMID: 8302217 [PubMed - indexed for MEDLINE]

Antonie Van Leeuwenhoek 1993-94;64(3-4):273-83

Related Articles, Links

Genetic interrelationships of proteolytic *Clostridium botulinum* types A, B, and F and other members of the *Clostridium botulinum* complex as revealed by small-subunit rRNA gene sequences.

Hutson RA, Thompson DE, Lawson PA, Schocken-Iturino RP, Bottger EC, Collins MD.

Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, UK.

The phylogenetic interrelationships of members of the *Clostridium botulinum* complex of species was investigated by direct sequencing of their 16S rRNA genes.

Comparative analysis of the 16S rRNA sequences demonstrated the presence of four phylogenetically distinct lineages corresponding to: i) proteolytic *C. botulinum* types A, B, and F, and *C. sporogenes*, ii) saccharolytic types B, E and F, iii) types C and D and *C. novyi* type A, and iv) type G and *C. subterminale*. The phylogenetic groupings obtained from the 16S rRNA were in complete agreement with the four divisions recognised within the the 'species complex' on the basis of phenotypic criteria.

PMID: 8085790 [PubMed - indexed for MEDLINE]

Related Articles, Links

Molecular cloning of the *Clostridium botulinum* structural gene encoding the type B neurotoxin and determination of its entire nucleotide sequence.

Whelan SM, Elmore MJ, Bodsworth NJ, Brehm JK, Atkinson T, Minton NP.

Division of Biotechnology, PHLS Centre for Applied Microbiology and Research, Salisbury, Wiltshire, United Kingdom.

DNA fragments derived from the *Clostridium botulinum* type A neurotoxin (BoNT/A) gene (botA) were used in DNA-DNA hybridization reactions to derive a restriction map of the region of the *C. botulinum* type B strain Danish chromosome encoding botB. As the one probe encoded part of the BoNT/A heavy (H) chain and the other encoded part of the light (L) chain, the position and orientation of botB relative to this map were established. The temperature at which hybridization occurred indicated that a higher degree of DNA homology occurred between the two genes in the H-chain-encoding region. By using the derived restriction map data, a 2.1-kb BglII-XbaI fragment encoding the entire BoNT/B L chain and 108 amino acids of the H chain was cloned and characterized by nucleotide sequencing. A contiguous 1.8-kb XbaI fragment encoding a further 623 amino acids of the H chain was also cloned. The 3' end of the gene was obtained by cloning a 1.6-kb fragment amplified from genomic DNA by inverse polymerase chain reaction. Translation of the nucleotide sequence derived from all three clones demonstrated that BoNT/B was composed of 1,291 amino acids. Comparative alignment of its sequence with all currently characterized BoNTs (A, C, D, and E) and tetanus toxin (TeTx) showed that a wide variation in percent homology occurred dependent on which component of the dichain was compared. Thus, the L chain of BoNT/B exhibits the greatest degree of homology (50% identity) with the TeTx L chain, whereas its H chain is most homologous (48% identity) with the BoNT/A H chain. Overall, the six neurotoxins were shown to be composed of highly conserved amino acid domains interceded with amino acid tracts exhibiting little overall similarity. In total, 68 amino acids of an average of 442 are absolutely conserved between L chains and 110 of 845 amino acids are conserved between H chains. Conservation of Trp residues (one in the L chain and nine in the H chain) was particularly striking. The most divergent region corresponds to the extreme carboxy terminus of each toxin, which may reflect differences in specificity of binding to neurone acceptor sites.

Related Articles, Links

The complete sequence of botulinum neurotoxin type A and comparison with other clostridial neurotoxins.

Binz T, Kurazono H, Wille M, Frevert J, Wernars K, Niemann H.

Institut für Medizinische Virologie der Justus-Liebig-Universität, Giessen,
Federal Republic of Germany.

The seven serologically different botulinum neurotoxins are highly potent protein toxins that inhibit neurotransmitter release from peripheral cholinergic synapses.

The activated toxins consist of the toxifying A-subunits (Mr approximately 50,000) linked by a disulfide bond to the receptor-binding BC-subunits (Mr approximately 100,000). We have established the complete sequence of botulinum neurotoxin type A (BoNT/A; 1,296 amino acid residues, Mr = 149,425) and a partial sequence of

botulinum neurotoxin type E (273 amino acid residues) as deduced from the corresponding nucleotide sequences of the chromosomally located structural genes. The promoter of the BoNT/A gene is inactive in *Escherichia coli*. Primer extension experiments indicated that initiation of transcription of the BoNT/A gene occurred 118

nucleotides upstream from the ATG codon. A comparison of the protein sequence revealed an overall identity of 33.8% to that of tetanus toxin. No significant similarity to other known proteins including ADP-ribosylating toxins could be detected. Three of the six histidine residues of the A-subunit of BoNT/A were found in the peptide sequence H223ELIHXXH230 within a domain of predicted alpha-helical secondary structure. This motif is also found in similar positions of the A-subunits of tetanus toxin and BoNT/E.

PMID: 2160960 [PubMed - indexed for MEDLINE]

P10844. Botulinum neuroto...[gi:399134]

Links

LOCUS BXB_CLOBO 1291 aa linear BCT 15-JUN-2002
DEFINITION Botulinum neurotoxin type B precursor (BoNT/B) (Bontoxilysin B).
ACCESSION P10844
VERSION P10844 GI:399134
DBSOURCE swissprot: locus BXB_CLOBO, accession P10844;
 class: standard.
 extra accessions:P10843,created: Jul 1, 1989.
 sequence updated: Jul 1, 1993.
 annotation updated: Jun 15, 2002.
 xrefs: gi: 144734, gi: 144735, gi: 40383, gi: 40384, gi: 407782,
 gi: 407783, gi: 98574, gi: 80489, gi: 80488, gi: 98573, gi: 98572,
 gi: 477374
 xrefs (non-sequence databases): HSSPP10845, MEROPSM27.002,
 InterProIPR000395, InterProIPR000130, PfamPF01742, PRINTSPR00760,
 ProDomPD001963, PROSITEPS00142
KEYWORDS Neurotoxin; Transmembrane; Hydrolase; Metalloprotease; Zinc.
SOURCE Clostridium botulinum
ORGANISM Clostridium botulinum
 Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae;
 Clostridium.
REFERENCE 1 (residues 1 to 1291)
AUTHORS Whelan,S.M., Elmore,M.J., Bodsworth,N.J., Brehm,J.K., Atkinson,T.
 and Minton,N.P.
TITLE Molecular cloning of the Clostridium botulinum structural gene
 encoding the type B neurotoxin and determination of its entire
 nucleotide sequence
JOURNAL Appl. Environ. Microbiol. 58 (8), 2345-2354 (1992)
MEDLINE 92384550
REMARK SEQUENCE FROM N.A.
REFERENCE 2 (residues 1 to 1291)
AUTHORS Szabo,E.A., Pemberton,J.M. and Desmarchelier,P.M.
TITLE Direct Submission
JOURNAL Submitted (~APR-1992)
REMARK SEQUENCE OF 35-245 FROM N.A.
 STRAIN=NCTC 7273
REFERENCE 3 (residues 1 to 1291)
AUTHORS Campbell,K.D., Collins,M.D. and East,A.K.
TITLE Gene probes for identification of the botulinal neurotoxin gene and
 specific identification of neurotoxin types B, E, and F
JOURNAL J. Clin. Microbiol. 31 (9), 2255-2262 (1993)

MEDLINE 94013372

REMARK SEQUENCE OF 633-993 FROM N.A.
STRAIN=NCTC 7273

REFERENCE 4 (residues 1 to 1291)

AUTHORS Dasgupta,B.R. and Datta,A.

TITLE Botulinum neurotoxin type B (strain 657): partial sequence and
similarity with tetanus toxin

JOURNAL Biochimie 70 (6), 811-817 (1988)

MEDLINE 89000987

REMARK SEQUENCE OF 1-44 AND 441-466.
STRAIN=657

REFERENCE 5 (residues 1 to 1291)

AUTHORS Schmidt,J.J., Sathyamoorthy,V. and DasGupta,B.R.

TITLE Partial amino acid sequences of botulinum neurotoxins types B and E

JOURNAL Arch. Biochem. Biophys. 238 (2), 544-548 (1985)

MEDLINE 85197963

REMARK SEQUENCE OF 1-16 AND 441-458.
STRAIN=OKRA

REFERENCE 6 (residues 1 to 1291)

AUTHORS Schiavo,G., Rossetto,O., Santucci,A., DasGupta,B.R. and
Montecucco,C.

TITLE Botulinum neurotoxins are zinc proteins

JOURNAL J. Biol. Chem. 267 (33), 23479-23483 (1992)

MEDLINE 93054694

REMARK IDENTIFICATION AS ZINC-PROTEASE.

REFERENCE 7 (residues 1 to 1291)

AUTHORS Schiavo,G., Benfenati,F., Poulain,B., Rossetto,O., Polverino de
Laureto,P., DasGupta,B.R. and Montecucco,C.

TITLE Tetanus and botulinum-B neurotoxins block neurotransmitter release
by proteolytic cleavage of synaptobrevin

JOURNAL Nature 359 (6398), 832-835 (1992)

MEDLINE 93063293

REMARK IDENTIFICATION OF SUBSTRATE.

COMMENT On or before Sep 14, 1993 this sequence version replaced gi:115189,
gi:115190.

This SWISS-PROT entry is copyright. It is produced through a
collaboration between the Swiss Institute of Bioinformatics and
the EMBL outstation - the European Bioinformatics Institute.
The original entry is available from <http://www.expasy.ch/sprot>
and <http://www.ebi.ac.uk/sprot>
-----.

[FUNCTION] BOTULINUS TOXIN ACTS BY INHIBITING NEUROTRANSMITTER
RELEASE. IT BINDS TO PERIPHERAL NEURONAL SYNAPSES, IS
INTERNALIZED

AND MOVES BY RETROGRADE TRANSPORT UP THE AXON INTO THE SPINAL CORD

WHERE IT CAN MOVE BETWEEN POSTSYNAPTIC AND PRESYNAPTIC NEURONS. IT

INHIBITS NEUROTRANSMITTER RELEASE BY ACTING AS A ZINC ENDOPEPTIDASE

THAT CLEAVES THE 76-GLN-|-PHE-77 BOND OF SYNAPTOBREVIN-2.

[CATALYTIC ACTIVITY] Limited hydrolysis of proteins of the neuroexocytosis apparatus, synaptobrevins, SNAP25 or syntaxin. No detected action on small molecule substrates.

[COFACTOR] Binds 1 zinc ion per subunit (By similarity).

[SUBUNIT] DISULFIDE-LINKED HETERODIMER OF A LIGHT CHAIN (L) AND A HEAVY CHAIN (H). THE LIGHT CHAIN HAS THE PHARMACOLOGICAL ACTIVITY,

WHILE THE N-AND C-TERMINAL OF THE HEAVY CHAIN MEDIATE CHANNEL FORMATION AND TOXIN BINDING, RESPECTIVELY.

[SUBCELLULAR LOCATION] Secreted.

[MISCELLANEOUS] THERE ARE SEVEN ANTIGENICALLY DISTINCT FORMS OF

BOTULINUM NEUROTOXIN: TYPES A, B, C1, D, E, F, AND G.

[SIMILARITY] BELONGS TO PEPTIDASE FAMILY M27.

FEATURES Location/Qualifiers

source	1..1291
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	/EC_number="3.4.24.69"
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	/note="BOTULINUM NEUROTOXIN B, LIGHT-CHAIN."
Region	30
	/gene="BOTB"
	/region_name="Conflict"
	/note="T -> M (IN REF. 4)."
Region	218
	/gene="BOTB"
	/region_name="Conflict"
	/note="R -> G (IN REF. 2)."
Region	225
	/gene="BOTB"

/region_name="Conflict"
 /note="A -> S (IN REF. 2)."
 Site 230
 /gene="BOTB"
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 /note="ZINC (CATALYTIC) (BY SIMILARITY)."
 Site 231
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 /note="BY SIMILARITY."
 Site 234
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 Bond bond(437,446)
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 /note="INTERCHAIN (PROBABLE)."
 Region 442..1291
 /gene="BOTB"
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 /note="BOTULINUM NEUROTOXIN B, HEAVY-CHAIN."
 Region 464
 /gene="BOTB"
 /region_name="Conflict"
 /note="S -> R (IN REF. 4)."

ORIGIN

1 mpvtinnfny ndpidnnnii mmeppfargt gryykafkit driwiipery tfgykpedfn
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 121 drrvpleefn tniasvtvkn lisnpgever kkgifanlii fgpgpvlnen etidigiqnh
 181 fasregfggi mqmkfcpeyv svfnvnqenk gasifnrrgy fsdpalilmh elihvlhgly
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 301 drlnkvlvci sdpnininiy knkfkdkykf vedsegkysi dvesfdklyk slmfgtetn
 361 iaenykiktr asyfsdslpp vkiknlldne iytieegfni sddmekeyr gqnkainkqa
 421 yeeiskehla vykiqmcksv kapgicidvd nedlffiadk nsfsddlskn erieyntqsn
 481 yiendfpine lildtdlisk ielpsenteltdfnvdvpv yekqpaikki fidentifqy
 541 lysqtfpldi rdlsstssfd dallfsnkvy sffsmdyikt ankvveaglf agwvkqivnd
 601 fvieanksnt mdkiadisli vpyiglalnv gnetakgnfe nafeiagasi llefipelli
 661 pvvgaflles yidnknkiik tidnaltkrn ekwsdmygli vaqwlstvnt qfytikeygmy
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 961 gwksisirgnr iwtliding ktksvffeyn irediseyin rwffvtitnn lnnakiying
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1081 seylkdfwgn plmynkeyym fnagnknsyi klkdsppvge iltrskynqn skyinyrdly
1141 igekfiirrk snsqsinddi vrkedyiyl dffnlqewrv ytykyfkkee eklflapisd
1201 sdefyntiqi keydeqptys cqllfkdee stdeigli hrfyesgivf eeykdyfcis
1261 kwylkevkrk pynlklgcw qfipkdegwt e

//

Revised: July 5, 2002.

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Oct 21 2002 11:56:56

Related Articles, Links

Cloning of a *Clostridium botulinum* type B toxin gene fragment encoding the N-terminus of the heavy chain.

Jung HH, Rhee SD, Yang KH.

Department of Life Science, Korea Advanced Institute of Science and Technology, Taejon, Korea.

Two lambda gt11 clones of the toxin gene of *Clostridium botulinum* type B were identified by the monoclonal antibody specific to the heavy chain of type B toxin.

Neither of the expressed fusion proteins from the lysates of lysogenic *E. coli* Y1089 showed any botulinal toxic activity. One of the clones hybridized to the oligonucleotide probe which was synthesized according to the amino acid sequence of N-terminus of heavy chain. The sequence analysis revealed that highly homologous regions in N-terminus of heavy chain exist among botulinum neurotoxins (type A, B) and tetanus toxin on the amino acid sequence level.

Related Articles, Links

Bacterial toxins.

Lubran MM.

Department of Pathology, Harbor-UCLA Medical Center, Torrance 90509.

Many bacterial toxins are proteins, encoded by the bacterial chromosomal genes, plasmids or phages. Lysogenic phages form part of the chromosome. The toxins are usually liberated from the organism by lysis, but some are shed with outer membrane proteins in outer membrane vesicles. An important non-protein toxin is lipopolysaccharide or endotoxin, which is a constituent of the cell wall of gram negative bacteria. Toxins may damage the eukaryotic cell membrane by combining with some structural component, or otherwise alter its function. Many toxins combine with specific receptors on the surface membrane, frequently glycoproteins or gangliosides, and penetrate the cell to reach their intracellular target. A common mechanism of entry is absorptive endocytosis. Many protein toxins have an A-B structure, B being a polypeptide which binds to the receptor and A being an enzyme. Many toxins are activated, either when produced by the bacterium or when bound to the membrane receptor, by proteases (nicking). An enzymatic process common to many toxins is adenosine diphosphate (ADP)-ribosylation of the adenylate cyclase regulatory proteins, leading to an increase in intracellular cyclic adenosine monophosphate (cAMP). This is the mechanism of action of cholera toxin. Diphtheria toxin catalyzes the transfer of ADP-ribose to elongation factor-2, inhibiting protein synthesis. Most toxins act on the target cells to which they bind, but tetanus toxin, and, to a lesser degree, botulinum toxin, ascend axons and affect more distant structures. Although many toxin effects caused by bacteria have been described, only a few toxins have been identified, characterized, and their mode of action determined at the molecular level. The best known of these are discussed.

Publication Types:

Review

Review, Tutorial

PMID: 3281562 [PubMed - indexed for MEDLINE]

Art Unit: 1645

Biochimie 1988, 70 (6):811-7

Related Articles by NCBI

Botulinum neurotoxin type B (strain 657): partial sequence and similarity with tetanus toxin.

Dasgupta,B R. , Datta,A.

Food Research Institute, University of Wisconsin, Madison 53706.

The type B neurotoxin (NT) isolated from *Clostridium botulinum* (strain 657) behaved as a mixture of single (unnicked) and dichain (nicked) proteins, both of Mr approximately 150 kDa.

When the dichain NT was reduced by mercaptoethanol, the two chains migrated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) as separate polypeptides of

Mr approximately 100 and 50 kDa that appeared similar to the heavy and light chains of other serotypes of botulinum NT. The N-terminal amino acid sequences of the two chains were

determined. They were as follows: light chain:

Pro-Val-Thr-Ile-Asn-Asn-Phe-Asn-Tyr-Asn-Asp-Pro-Ile-Asp-Asn-Asn-Asn-Ile- Ile-Met -

Art Unit: 1645

Met-Glu-Pro-Pro-Phe-Ala-Arg-Gly-Met-Gly-Arg-Tyr-Tyr-Lys-Ala-Phe-Lys-Ile- Thr-Asp -
Arg-Ile-Trp-Ile-; and heavy chain:

Ala-Pro-Gly-Ile-X-Ile-Asp-Val-Asp-Asn-Glu-Asp-Leu-Phe-Phe-Ile-Ala-Asp-Ly s-Asn-
Ser-Phe-Arg-Asp-Asp-Leu-. These two sequences matched exactly with those of the light and
heavy chains of type B NT (strain Okra) of which only 16 and 18 residues were known (J.
Biol. Chem. (1985) 260, 10461). The above sequences were different from those of type A NT.

Immunoprecipitation reactions of type B NT isolated from strains 657 and Okra were
indistinguishable against polyclonal anti-type B NT serum. These two preparations did not
produce

precipitin reactions with polyclonal anti-type A NT serum.(ABSTRACT TRUNCATED AT
250 WORDS)

MedlineID: 89000987

PMID: 3139097

ISSN: 0300-9084

Art Unit: 1645

Eur J Biochem 1988 Nov 15;177(3):683-91

Related Articles, Links

Involvement of the constituent chains of botulinum neurotoxins A and B in the blockade of neurotransmitter release.

Maisey EA, Wadsworth JD, Poulain B, Shone CC, Melling J, Gibbs P, Tauc L, Dolly JO.

Department of Biochemistry, Imperial College, London, England.

1. The abilities of botulinum neurotoxins, types A and B (single and two-chain forms) to inactivate an intraneuronal component required for transmitter release were quantified in a phrenic-nerve-diaphragm preparation, cerebrocortical synaptosomes or the buccal ganglion of *Aplysia californica* and compared with the mouse toxicity assay. 2. Homogeneous preparations of the individually renatured polypeptide chains of both toxin types showed low residual toxicity in the whole animal and had no

Art Unit: 1645

effect on neurotransmission in all three systems, when tested singly. 3. Mixtures of individually renatured heavy chain, from type A or B, and either light chain proved very effective in blocking the evoked release of acetylcholine when bath-applied to the buccal ganglion of *Aplysia* whilst they were relatively inactive on mammalian nerve terminals, indicating a less efficient uptake of the polypeptides in the latter.

4. When renatured together, the homologous, but not the heterologous, chains of each toxin type yielded toxic, disulphide-linked two-chain species. 5. A role for the heavy chain alone in acceptor recognition and membrane translocation was implicated by the blockade of acetylcholine release produced when light chain was applied to a ganglion of *Aplysia* previously bathed in heavy chain and washed extensively. No blockade was observed when the order of application of the two chains was reversed. 6. These findings are discussed in the context of the intracellular requirement for both the constituent toxin chains for toxicity, and in the apparent need for these chains to be linked via a disulphide bond for uptake in rodents but not in *Aplysia*.

Related Articles, Links

Gene probes for identification of the botulinal neurotoxin gene and specific identification of neurotoxin types B, E, and F.

Campbell KD, Collins MD, East AK.

Reading Laboratory, Institute of Food Research, Agriculture and Food Research Council, United Kingdom.

A polymerase chain reaction method was developed for the specific detection of the botulinum neurotoxin (BoNT) gene of *Clostridium botulinum*. Degenerate oligonucleotide primers, designed from the nucleotide sequence of the heavy chain of the BoNT gene, amplified a specific fragment of approximately 1.1 kb from strains of *C. botulinum* toxin types A, B, E, F, and G and neurotoxin-producing strains of *Clostridium barati* and *Clostridium butyricum*, but no fragment was obtained from nontoxigenic strains. The fragments amplified from several strains of *C. botulinum* types B, E, and F were cloned in *Escherichia coli* and their nucleotide sequences were determined. Sequences within this region were used to design oligonucleotide probes specific for BoNT type B (BoNT/B), BoNT/E, and BoNT/F genes. An additional probe was designed for the detection of the BoNT/F gene of *C. barati*, which differed in sequence from BoNT/F genes of both proteolytic and nonproteolytic strains of *C. botulinum*.

PMID: 8408542 [PubMed - indexed for MEDLINE]